4-12-2013

Two NCS1 transporters of the moss Physcomitrella patens share substrate specificities with other members of the NCS1 family but express novel phenotypes and distinct transport profiles

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Two NCS1 transporters of the moss *Physcomitrella patens* share substrate specificities with other members of the NCS1 family but express novel phenotypes and distinct transport profiles

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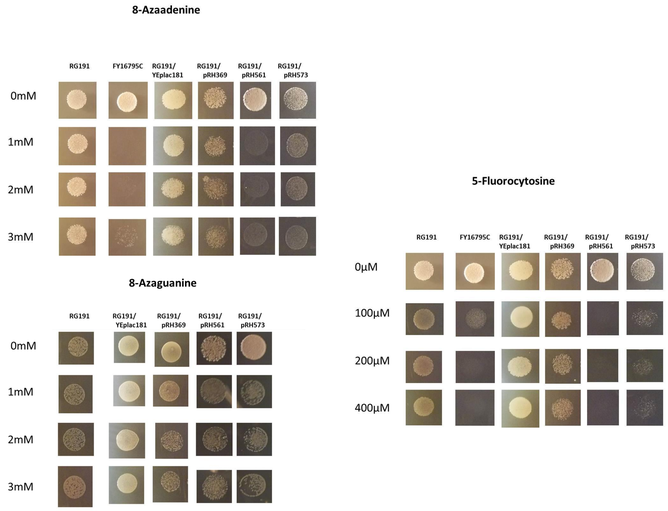
Abstract

The two genes PpNCS1A and PpNCS1B of the moss *Physcomitrella patens* are nucleobase transporters and putative members of the purine-related transporter (PRT) or nucleobase:cation symporter 1 (NCS1) family. Previously characterized members of this family include the uracil transporter (FUR 4) of *Saccharomyces cerevisiae*, the adenine-guanine-hypoxanthine-cytosine transporter (FCY2) of *S. cerevisiae* and the recently characterized adenine-guanine-uracil transporter (AtNCS1) of *Arabidopsis thaliana*. The two NCS1 genes of *P. patens* were cloned into yeast expression vectors and expressed in yeast strains lacking their own NCS1 genes. Transport profiles of the two P. patens NCS1 genes were discovered by radiolabeled nucleobase uptake and competition assays, and toxic analog growth studies. Interestingly, the two genes exhibit overlapping yet distinct solute specificities. PpNCS1A transports adenine, cytosine and uracil, but not guanine, while PpNCS1B transports adenine, cytosine and guanine, but not uracil. Future work will confirm the results presented here by yeast growth studies on media containing a sole nitrogen source, and kinetic parameters will be determined by radionucleobase homologous and/or heterologous competition assays.

Introduction

Nucleotides are the building blocks of nucleic acids, which constitute one of the four major classes of biological molecules. Nucleotides may be synthesized de novo, or derived via salvage pathways; pathways involved in nucleotide metabolism are ancient, highly compartmentalized, and often conserved. Crucial to the proper function of these pathways are nucleobase transporters, which facilitate the movement of nucleobases and their derivtives across selective membranes. Nucleobase transporters have variable substrate specificities and transport profiles, which are often representative of the transporter family to which they belong. Among plants, for example, members of the Purine Related Transporter (PRT) or Nucleobase: Cation Symporter 1 (NCS1) family are known to transport adenine, guanine, uracil and cytosine. The current study seeks to determine the transport profile of two putative members of the NCS1 family found in the moss *Physomitrella patens*.

Toxic Analog Growth Assays



Yeast transformed with plasmid pRH561 (PpNCS1A) or plasmid pRH573 (PpNCS1B) exhibits sensitivity to the toxic nucleobase analogs 8-azaadenine, 8-azaguanine and 5-fluorocytosine

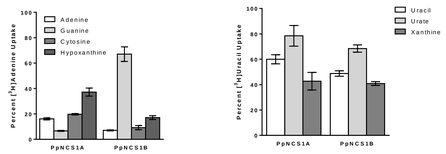
Materials and Methods

***S. cerevisiae* strains RG191 (Met a leu2Δ met15Δ ura3Δ fcy2 Δ) and ATTC: 4003158 (Mat a his3Δ leu2Δ met15Δ ura3Δ fur4Δ) were transformed using the Li/Ac/SS carrier method with the plasmids pRH561 (PpNCS1A in vector pRG399) and pRH573 (PpNCS1B in vector pRG399). The strains were allowed to grow overnight in liquid Synthetic Complete medium (.66% yeast nitrogen base, 2% gluose, .002% each his, met, ura) at 30°C and allowed to reach an OD_{600} value of .6. Strains were then plated on solid SC media containing increasing concentrations of toxic nucleobase analogs and allowed to grow for two days at 30°C. Yeast expressing no NCS1 transporter gene, PpNCS1A or PpNCS1B was incubated for 5 minutes with .25 µM ^{3}H Adenine, 1 µM ^{3}H Guanine, 1 µM ^{3}H Uracil, .5 µM ^{3}H Hypoxanthine or 1 µM ^{3}H Xanthine; cell and isotope solution was filtered, washed with sterile ddH_2O, and radioactivity of filters was measured by a scintillation counter.**

Substrate Specificities and Affinities

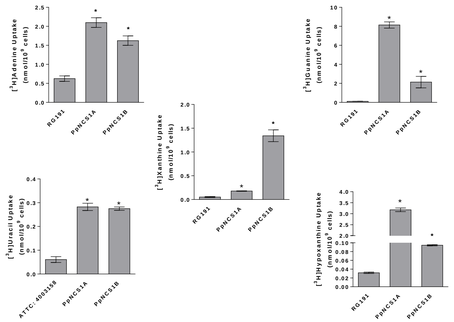
	PpNCS1A	PpNCS1B
Uptake of ^{3}H Adenine	++	++
Uptake of ^{3}H Guanine	++	+
Uptake of ^{3}H Uracil	++	++
Uptake of ^{3}H Hypoxanthine	++	+
Uptake of ^{3}Xanthine	+	++
Competition: Adenine/^{3}HA	++	++
Competition: Guanine/^{3}HA	++	+
Competition: Cytosine/^{3}HA	++	++
Competition: Hypoxanthine/^{3}HA	++	+
Competition: Uracil/^{3}HU	+	+
Competition: Urate/^{3} HU	-	-
Competition: Xanthine/^{3}HU	+	+
Sensitivity to 8-azaadenine	++	+
Sensitivity to 8-azaguanine	++	+
Sensitivity to 5-fluorocytosine	+	+

Heterologous Competition



Yeast expressing either PpNCS1A or PpNCS1B was incubated for 5 minutes with ^{3}H Adenine or ^{3}H Uracil alone or together with a cold competitor present at a concentration 10,000 fold that of the radiolabeled nucleobase; uptake of ^{3}H Adenine or ^{3}H Uracil in the presence of a competitor was calculated as a percentage of uptake of ^{3}H Adenine or ^{3}H Uracil alone

Radionucleobase Uptake



Yeast expressing PpNCS1A exhibits significant uptake of ^{3}H Adenine (p=.0005), ^{3}H Guanine (p=.0001), ^{3}H Uracil (p=.0004), ^{3}H Hypoxanthine (p=.0001) and ^{3}H Xanthine (p=.0004); Yeast expressing PpNCS1B exhibits significant uptake of ^{3}H Adenine (p=.0022), ^{3}H Guanine (p=.0288), ^{3}H Uracil (p=.0001), ^{3}H Hypoxanthine (p=.0001) and ^{3}H Xanthine (p=.0005)

Uptake of ^{3}H Adenine by yeast expressing PpNCS1A or PpNCS1B decreased in the presence of cold adenine, guanine, cytosine and hypoxanthine competitors; uptake of ^{3}H Uracil by yeast expressing PpNCS1A or PpNCS1B decreased in the presence of cold uracil and xanthine competitors. The presence of cold urate did not substantially decrease uptake of ^{3}H Uracil

Conclusion

The putative NCS1 genes belonging to the moss *P. patens* exhibit identical solute specificities, but differing affinities for those compounds. Additionally, their transport profiles do not exactly match any of the transport profiles of NCS1 family gene members previously characterized.